

Microbiome Mediates Development of PTSD and Resilience

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Abstract

Identification of youth at risk for post-traumatic pathology is critical for public health, medicine, and social policy but research has not yet succeeded in pinpointing biomarkers that can distinguish the post-traumatic from the resilient profile in contexts of trauma. As trauma alters the microbiome with lasting effects on the host, the current longitudinal, multi-measure, cross-species study sought to outline the microbial signature of post-traumatic stress disorder (PTSD). We followed a unique trauma-exposed cohort for 15 years, from early childhood to adolescence, repeatedly assessing post-traumatic symptomatology. Gut microbiome composition and diversity characterized post-traumatic pathology, distinguished youth with PTSD from resilient individuals, and mediated the continuity of post-traumatic disorder. Mother-child microbial synchrony was reduced in cases of PTSD, suggesting that diminished microbial concordance among family members may index susceptibility to post-traumatic illness. Germ-free mice transplanted with PTSD microbiomes compared with those receiving resilient microbiomes exhibited anxious behavior. Our findings provide causative evidence that the microbial trauma profile is at least partially responsible for the trauma-related phenotype and highlight microbial underpinnings of resilience. Our results suggest that the microbial ecology may serve as additional biological memory of early life stress and underscore the potential for microbiome-related diagnosis and treatment following trauma exposure.

Main Text

Since the first diagnosis of post-traumatic-stress disorder (PTSD) in veterans of World War I who came back from the trenches physically intact but mentally impaired, the early identification of individuals at risk of developing post-traumatic pathology from those exposed to the same trauma who are more resilient has been an important topic in psychiatry, public health, education, governance, and the military¹. It has been suggested that the post-trauma phenotype is underpinned by susceptibility of specific neural and biological systems and typically involves exposure to heightened levels of stress in early life when such systems undergo rapid maturation². However, due to the correlative nature of human neurobiological research, lack of longitudinal follow-up studies that describe how early stress shapes post-traumatic illness, and difficulty to find sufficiently large cohorts exposed to the same trauma over lengthy epochs, research on the pathophysiology of PTSD has not been able to establish causal links between specific biological indices and the post-trauma phenotype nor pinpoint biomarkers that can distinguish the post-traumatic from the resilient profile in contexts of trauma³.

The main hypothesis guiding the current longitudinal, cross-generational, multi measure study validated by a germ-free (GF) mouse model, is that the microbiome plays a key role in the post-trauma pathology and mediates the development of PTSD versus resilience from early childhood. The microbiome is thought to mediate, in part, the development of trauma-related phenotypes⁴⁻⁶. Animal studies have shown that antibiotics given the first days of life carry long term effects on murine behavior⁷, including anxiety-like and aggressive behavior and impaired sociality. Human studies have similarly pointed to the

effects of early life stress on the developing microbiome⁸ and postulated that such impact may mediate the long-term effects of early life stress on stress-related pathologies in later life⁹. In the current study, we utilized a "natural experiment" context that affords a rare perspective on key issues related to the role of the microbiome in post-trauma. A unique cohort of children and mothers exposed to chronic mass trauma were followed for 15 years, from early childhood to late adolescence, and post-traumatic symptomatology and maternal caregiving were repeatedly assessed to elucidate how the microbiome mediates the development of PTSD following chronic early life stress. We focused on three features of the microbiome: composition, diversity, and mother-child microbial synchrony in light of research linking stress-related pathologies with alterations in these components⁴. Our aim was to identify the microbial underpinnings of post-trauma and resilience and examine pathways by which the microbiome mediates the consolidation of PTSD across development. Finally, utilizing GF mice implanted with feces from PTSD and resilient participants, we sought to tap into mechanisms linking microbiome with PTSD.

During 2003-2004, we recruited 148 mother-child dyads from Sderot, Israel, a town located 10km from the Gaza border and exposed to continuous war-related trauma for over two decades, and an 84-dyad comparison (control) group from towns within the greater Tel-Aviv, Israel area with matched sociodemographic variables¹⁰. In early childhood, children's PTSD was diagnosed by trained clinicians¹¹. In early childhood, late childhood, and early adolescence, age-appropriate interactions between mothers and children were videotaped and maternal sensitivity was coded offline to create a mean score of maternal sensitive caregiving across development¹². In late adolescence, trained clinicians evaluated mothers' and adolescents' psychiatric condition¹³ and fecal samples were collected from mothers and adolescents (Fig. 1a).

Trauma exposure increases PTSD and reduces sensitive caregiving. In early childhood, war-exposed children had significantly more PTSD symptoms compared to controls ($t_{(82)}=-6.03$ $p<0.001$) and greater likelihood of clinically-diagnosed PTSD ($\chi^2_{(1)}=84.00$, $p<0.001$). In late adolescence, exposed adolescents had more psychological symptoms, ($t_{(82)}=-3.03$ $p=0.001$) and greater likelihood of clinically-diagnosed PTSD ($\chi^2_{(1)}=12.85$, $p<0.001$). Trauma-exposed mothers exhibited significantly less sensitive caregiving across development compared with controls ($t_{(82)}=2.04$, $p=0.044$). These results indicate that trauma-exposed children suffer not only from higher rates of PTSD and related psychiatric symptoms but also receive less optimal caregiving, which further exacerbates the degree of toxic stress experienced by children exposed to harsh, unpredictable environments.

PTSD is reliably identified using the microbiome profile. To validate the association between war-exposure, its psychiatric sequelae, and the gut microbiome, we first tested whether the microbiome can be used to characterize PTSD versus resilience. We were able to classify both war-exposure and PTSD in late adolescence based solely on microbial community composition for the entire sample (mothers and adolescents), as well as for the adolescents and mothers separately (Fig. 1b). We were also able to characterize adolescents' psychological symptoms on the basis of their microbial community using an XGBOOST gb linear regression¹⁴ ($r=0.59$, $R^2=0.28$, $p=0.004$). Adolescents' war-exposure and PTSD

diagnosis were both strongly linked with their microbial profile, whereas among mothers, the microbial profile was more closely associated with exposure status than with maternal PTSD (Fig. 1c, Extended Data Fig. 1a-d). Classifier models identified 8 important genera (4 positively and 4 negatively correlated with PTSD) that differ in abundances in the youth PTSD group compared to those without PTSD (Fig. 1d), and 9 important genera characterized youth exposed to war compared to controls (Extended Data Fig. 1e). Our results are in line with previous studies on the associations between the microbiome and mental health. For instance, *Dialister* - which has been previously shown to decrease in cases of PTSD and chronic depression¹⁵ and *Veillonella* that is over-represented in individuals with depression¹⁶ - were found here to be associated with PTSD.

PTSD reduces mother-child microbial synchrony. To test microbial synchrony, the concordance between mother and her own child's microbiome composition, we examined the distances between mothers' and adolescents' microbiome diversity using the Euclidean norm of the log-normalized microbiome¹⁷ (Fig. 1e, referred to as "synchrony"). Overall, adolescents were more similar to their own mothers as compared to other mothers as well as all mothers in our cohort (Fig. 1f). Comparing microbial synchrony in dyads of adolescents with and without PTSD revealed that adolescents with PTSD had microbial diversity less similar to that of their mothers, as compared with dyads without PTSD diagnosis ($t_{(32)}=1.97, p=0.03$). Comparing dyadic microbial synchrony between dyads with and without maternal PTSD did not reveal a significant effect (Fig. 1g). Similarly, there was no difference in microbial synchrony between exposed and control dyads, suggesting that the decreased microbial synchrony in cases of adolescent PTSD is diagnosis-specific. Microbial synchrony was marginally correlated with youth psychological symptoms ($SCC=0.36, p=0.083$, Fig. 2a). These findings indicate that mother-child microbial synchrony is impaired when adolescents suffer from PTSD. These results are in line with research indicating decreased mother-child hormonal synchrony in cases of childhood adversities and negative developmental outcomes^{18,19}

To characterize the gut microbiome's association with trauma and its sequelae, we compared microbial richness (alpha-diversity) between participants from the exposed vs. control group and from those with PTSD vs. those without a psychiatric diagnosis in late adolescence. Participants from the exposed group had significantly lower levels of microbial richness compared with controls (Shannon's diversity index, $t_{(63)}=2.54, p=0.015$). No significant difference was found between participants with and without PTSD at late adolescence ($t_{(63)}=0.582, p=0.417$); however, we did find significant correlations between adolescents' microbial diversity and their psychological symptoms ($SCC=-0.52, p=.005$, Fig. 2a). These results are consistent with studies reporting lower alpha diversity among individuals with anxiety and depressive disorders^{20,21} and with research indicating negative correlation between alpha diversity and psychiatric symptom severity²¹, childhood adversity, and disrupted caregiving²².

Gut microbiome mediates development of PTSD. Mothers' sensitive caregiving across development, as repeatedly assessed from early childhood to adolescence, was linked with youth alpha-diversity ($SCC=0.38, p=0.026$), highlighting the long-term impact of maternal sensitive attunement,

empathy, and affect matching on the maturation of diverse microbiota (Fig. 2a). A negative correlation was found between PTSD symptoms in early childhood and Shannon's alpha diversity more than a decade later (SCC=-0.49, $p=0.003$, Fig. 2a), suggesting that early-childhood PTSD may play a role in the consolidation of the microbiome. We next divided our cohort into three groups based on early-life psychopathology using PTSD diagnosis in early childhood: (a) PTSD - war-exposed children with clinically-diagnosed PTSD (Exp-PTSD), (b) Resilience - a group of children exposed to trauma but without post-traumatic symptoms or any other psychiatric diagnosis (Exp-ND), and (c) Control - healthy controls who were screened for exposure to any other trauma (No-TR). A Kruskal-Wallis test showed difference in late adolescents' alpha diversity ($\chi^2_{(2)}=6.72$, $p=0.035$), and post hoc comparisons revealed that the Exp-PTSD group had significantly lower microbial diversity compared with the Exp-ND and No-TR groups ($U=42.00$, $Z=-2.10$, $p=0.036$ and $U=27.00$, $Z=-2.13$, $p=0.034$, respectively; fig. 2b). These findings highlight the long-term impact of early post-trauma and resilience on maturation of the microbiome in the context of chronic trauma exposure. The longitudinal associations between maternal sensitive caregiving across development, early-childhood PTSD, and later microbiome diversity are consistent with findings in animal models^{23,24}.

We utilized two structural models to test the mediating role of the microbiome in identifying the continuity of trauma-related symptoms from early childhood to late adolescence. In the first model, young children's PTSD symptoms were used to predict psychological symptoms in late adolescence, with microbial diversity and microbial synchrony as mediators, controlling for initial age and gender. Early-childhood PTSD predicted both alpha diversity and microbial synchrony. Microbiome diversity, in turn, was linked with late-adolescence symptomatology; however, the path between microbial synchrony and adolescents' psychological symptoms was not significant. The model had an excellent fit to the data. We also ran this model without missing data imputation using the PROCESS macro for SPSS²⁵ and found similar results in terms of effect sizes and significance (see Extended Data Fig. 1f). This model confirms our key hypothesis that the gut microbiome mediates the pathway from PTSD in early life and post-traumatic symptomatology in adolescence in contexts of chronic trauma (Fig. 2c).

Our second model predicted adolescents' psychological symptoms from early childhood PTSD and adolescent microbiome composition. We ran three alternative models: PTSD diagnosis and the abundances of all microbial taxa grouped at the genus level, only PTSD diagnosis, and only microbiome abundances. We found that the model that predicted adolescents' psychological symptoms from both PTSD diagnosis and the microbiome was $2.476 \cdot e+28$ (F-score) and $7.519 \cdot e+28$ (F-score) times better than the models of only PTSD or only microbiome, respectively. In both models, the genus *Dialister* was negatively correlated and the genus *Veillonella* was positively correlated with PTSD (Fig. 1d, 2d).

Fecal transplant to a germ-free mouse model ascertains microbial involvement in the PTSD and resilient phenotypes. To pinpoint the microbial underpinnings of PTSD, we conducted a fecal microbiota transplantation (FMT) experiment in germ-free mice. FMT donors were all trauma-exposed adolescents in two groups; (a) PTSD: adolescents with a clinical PTSD diagnosis, and (b) No Diagnosis: adolescents without clinical diagnosis (Fig. 3a). This allowed us to ensure that any phenotype transfer

observed was PTSD-related rather than trauma-exposure derived. Following FMT and subsequent colonization, mouse behavior was assessed using an elevated plus maze (EPM)²⁶. Fecal samples were collected prior to the behavioral test to examine compositional differences between the groups.

We did not find significant differences in alpha-diversity between the two mouse groups; however, there were significant differences in beta-diversity (PERMANOVA: Jaccard, $p=0.038$; Bray-Curtis, $p=0.039$; Fig. 3b,c). ANCOM²⁷ analysis revealed that *Clostridium ramosum* was significantly more abundant in mice receiving FMT from adolescents with PTSD (Fig. 3d). This species is known to stimulate serotonin release from enterochromaffin cells in the colon²⁷, and serotonin has been implicated in anxiety and PTSD in mice and humans²⁸. The EPM test revealed that FMT of trauma-exposed youth with PTSD to germ-free mice increased anxious behavior: these mice entered the open arms less frequently compared to those receiving transplants from No Diagnosis exposed adolescents ($t_{(14)}=2.29$, $p=0.04$, Fig. 3e). Phenotypic transfer of anxiety following FMT confirms the role of the microbiome and/or associated metabolites in the PTSD pathology.

Conclusion

Our multigenerational, longitudinal, cross-species study embedded within a unique context of chronic trauma marks the first integrative attempt to describe the gut microbiome's role in the development of PTSD. We followed mother-offspring dyads from a zone of chronic war-related trauma and a matched non-exposed region over 15 years to determine involvement of the trauma-exposed microbiome in post-traumatic illness and examine whether microbial synchrony among cohabitating genetically-related individuals may be implicated in the pathophysiology of PTSD. Using machine learning we identified a trauma-specific microbial profile that distinguished youth exposed to chronic trauma who are at greater risk for developing post-traumatic pathology from those exposed to the same trauma who were more resilient. Furthermore, our findings on the decreased microbial synchrony among mother-child pairs in cases of PTSD can be of assistance in classification of the disorder. The early identification of youth at greater risk in the face of trauma has been a key issue in medicine, public health, allocation of government resources to trauma-stricken regions, and the recruitment of young adults into positions that involve hazards, such as police, military, fire-fighting, or emergency medicine and our findings may contribute to the formation of microbiome-specific tools that can distinguish the post-traumatic from the resilient profiles in trauma-inducing contexts.

PTSD was underpinned by a distinct microbiota composition, diversity, and synchrony profiles, and microbiome diversity mediated the pathway from post-traumatic symptomatology in early childhood to PTSD pathology 15 years later, demonstrating, for the first time, microbial mediation in the trajectory of a distinct psychiatric disorder. Although our study does not present causality in predicting psychopathology from microbial alteration in humans, we were able to provide causal evidence for the critical role of the gut microbiome in the development of post-trauma versus resilience using a germ-free mouse model.

Our results expand current knowledge on the mechanisms underlying the effects of early trauma exposure and parental caregiving on the gut microbiota. While involvement of the gut-brain axis in affective and behavioral processes has been reported²⁹, our study is the first to examine the link between microbiome characteristics and PTSD in humans as well as the first to use fecal transplantation in germ-free rodents to pinpoint the microbiome signature of a distinct psychiatric disorder. Our results may suggest that beyond its direct effect on the development of PTSD, the gut microbiome may form an additional layer of biological memory that retains the impression of previous events, particularly those occurring in early life. Such memory may define one pathway by which stress experienced in early life induces post-traumatic pathology many years later when stressful events re-occur by shaping the child's lifetime stress response, thereby charting a microbiome-specific pathway for the life-long impact of early-life stress. Much further research is required to pinpoint the involvement of the gut microbiome in the consolidation of psychiatric illness, the increased susceptibility following early life stress, and the trajectories of resilience in contexts of trauma toward the construction of novel and disease-specific microbiome-based interventions.

Methods

Human study cohort

Participants

Participants were recruited in 2004-2005 and included 232 children and their mothers (47.6% males and 47.1% firstborns). Of these, 148 mother-child dyads were geographically proximate, living in the same frontline neighborhoods in Sderot, Israel, and comprised the trauma-exposed group. The second group of 84 dyads was recruited from towns within the greater Tel Aviv, Israel area with similar sociodemographic variables to the town of Sderot. These dyads were not exposed to chronic war-related events and were considered the control group. Control children were matched to the exposed group with respect to age, sex, birth order, maternal and paternal age and education, maternal employment, and marital status³⁰. Before recruitment, control families were screened for major traumatic events in the child's life (e.g., motor vehicle accidents), and those reporting such trauma were excluded. Children in both groups were screened for records of physical abuse or neglect. Children were followed 5 times from early childhood to late adolescence at the following developmental stages: early childhood: $n=232$, $M_{age}=2.76$ years \pm 0.91; middle childhood: $n=210$, $M_{age}=7.68$ years \pm 0.7; late childhood: $n=177$, $M_{age}=9.3$ years \pm 1.41; early adolescence: $n=111$, $M_{age}=11.66$ years \pm 1.23; late adolescence: $n=84$, $M_{age}=16.13$ years \pm 1.22. Attrition at early stages was mainly related to the inability to locate families or families moving out of Sderot. No sociodemographic differences were found between continuing or non-continuing families. The study was approved by the University's Institutional Review Board, and all parents signed informed consent.

Procedure

In early childhood, late childhood and late adolescence, home visits were held by trained clinical psychologists. Middle childhood included phone-based interviews, and at early adolescence, participants came to the laboratory. In early childhood, 10 minutes of mother-child free play interactions were videotaped. The experimenter placed a box of preselected toys in front of the mother, and instructions were "Play with your child as you normally do"³¹. In late childhood, we used the well-validated positive interaction paradigm in which mother and child are given seven minutes to plan the "best day ever" to spend together³¹. In early adolescence, dyads visited the laboratory and were asked to play an "Etch a Sketch" game together for 7 minutes. Each partner controlled one knob and the two collaborated in drawing a joint picture³². All three interactions were coded with the well-validated coding interactive behavior system (CIB)¹². In late adolescence, dyads were revisited at home by trained clinical psychologists who conducted psychiatric interviews and left a stool kit. During the home visits sterile test tubes and instructions were given and subjects were instructed to use the sterile spoon and sterile 50 mL tubes to collect fresh stool samples (FloraPrep, Admera Health, South Plainfield, NJ). Participants kept the tubes in a cold dark place for 3-7 days until a research assistant collected the samples. Samples were kept frozen at -80°C until analyzed using 16S rRNA gene sequencing, as previously described^{17,33,34}.

Measures

Child PTSD in early childhood: PTSD was assessed by trained clinicians according to psychiatric classification criteria for early childhood. Mothers were interviewed about their children's post-traumatic symptoms based on the diagnostic criteria for young children proposed by The Diagnostic Classification of Mental Health and Developmental Disorders of Infancy and Early Childhood (DC:0-5)¹¹. First, mothers were asked in detail about the nature of the trauma, the level of exposure of the toddler and his family, a description of the details of the incident, whether any of the family members were directly harmed and the expression of the toddler's fear, terror and general emotional response to the traumatic event. Next, mothers rated the child's post-traumatic symptoms. The experimenter read to the mother a list of 58 items describing symptoms of re-experiencing, avoidance and withdrawal, over-arousal, and new fears and aggressions. Each of the symptoms was coded on a 3-point scale that referred to the frequency of symptoms: 1- does not occur, 2- describes a symptom that occurred with a low frequency (less than once a week) and 3 describes a high frequency of the symptom (at least two-three times a week). PTSD was diagnosed based on the DC:0-5 criteria which include: 1. Exposure to a traumatic event and expression of fear following it. 2. Expression of at least one symptom of re-experience, one symptom of avoidance, and two symptoms of hyper-arousal. 3. In light of the debate in the literature, to qualify for a full-blown PTSD, the child had to present at least one symptom from the "new fears and aggression" category.

Child psychiatric diagnosis in adolescence: The Developmental and Well-Being Assessment (DAWBA) was used to diagnose adolescents' Axis-I PTSD and internalizing disorder in early adolescence. The DAWBA is a structured interview generating ICD-10 and DSM-IV/DSM-5 psychiatric diagnoses in 5- to 17-year-old children¹³. The DAWBA, administered to mothers, is well-validated, including a large

epidemiological study in Israel³⁵. The DAWBA was administered by clinical psychologists and supervised by the same child psychiatrist, blind to all other information. Cases were conferred with reliability exceeding 85%. Severe PTSD symptoms without full-blown PTSD diagnosis were included only if accompanied by anxiety and/or depression diagnoses.

Child behavioral and emotional problems: At late adolescence, mothers completed the Child Behavior Checklist 6–18 years (CBCL)³⁶, a well-validated questionnaire of a child's psychological symptoms yielding externalizing, internalizing, and total behavior symptom scores. The CBCL includes 113 items each rated on a 3-point scale ranging from 0 (never applies) to 2 (almost always applies), later clustered into a total score. The CBCL is the most widely used instrument for assessing behavior and emotional problems in children with established reliability and validity³⁷.

Maternal psychiatric diagnosis at adolescence: Mothers were interviewed and diagnosed using the Structured Clinical Interview for Diagnostic Manual of Mental Disorder and Disease-Fourth Edition (DSM–IV) Axis-I Disorders (SCID-I)³⁸. This is a semi-structured, clinician-administered diagnostic interview that includes modules corresponding to major DSM psychiatric classifications with high reliability and validity³⁹.

Behavioral variables: Mother-child interactions were coded using our well-validated Coding Interactive Behavior Manual (CIB)¹². The CIB is a global rating system including multiple scales, each ranging from 1 to 5, that are integrated into theoretically meaningful constructs by averaging the relevant scales. The CIB has been validated in a large number studies globally across multiple cultures, ages, and psychopathologies with good psychometric properties^{40,41}. The construct of maternal sensitivity was used, which includes the following codes: maternal acknowledgment of child's social communication, consistent caregiving style, appropriate range of affect, recognition of child's distress or changing state, expansion of child's nonverbal or verbal signals, creativity, maternal supportive presence, and affectionate touch. The maternal sensitivity construct was calculated as the average from the interactions in early childhood, late childhood and early adolescence. Coding was conducted by two trained coders, blind to any other information, and exceeded 90% reliability on 20% of interactions for all coded behaviors ($k=.82$, range=.74–.95). Cronbach's α values for maternal sensitivity were 0.91, 0.87, 0.89, for early childhood, late childhood and early adolescence respectively.

Health and diet: Mothers were asked prior to scheduling the home visits in late adolescence whether they or their child had taken antibiotics in the past six months and information regarding digestive problems, bowel diseases, COVID-19 and any other medication use was collected. No significant difference was found between war-exposed and control participants in these variables. Furthermore, participants were asked to self-report if they are vegetarians or not. Home visits were scheduled only if the mother and child did not take antibiotics three weeks before the visit, did not suffer from any infection, felt healthy and were at least one week after their last menstrual period.

Microbiome sequencing

DNA extraction and 16S rRNA gene amplification and sequencing were performed for both human and mouse samples as follows: DNA was extracted from all fecal samples, using a PureLink Microbiome DNA Purification Kit (Thermo Fisher, Waltham, MA) according to the manufacturer's instructions and following a 2-minute bead beating step. The V4 region of the bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the 515F (AATGATACGGCGACCACCGAGATCTACACGCT) barcoded and 806R (TATGGTAATTGTGTGYCAGCMGCCGCGGTAA) primers⁴². Each reaction contained a final concentration of 0.04% of each primer and 0.5% PrimeSTAR Max DNA Polymerase (Takara-Clontech, Shiga, Japan) in 50µl total volume. PCR reactions were carried out by 30-35 cycles of denaturation (95°C), annealing (55°C) and extension (72°C), with final elongation at 72°C. PCR products were purified using KAPA Pure magnetic beads (Roche, Pleasanton, CA) and quantified using the Picogreen dsDNA quantitation kit (Invitrogen, Carlsbad, CA). Samples were then pooled in equal amounts, loaded on 2% agarose E-Gel (Thermo Fisher, Waltham, MA), purified, and sequenced using the Illumina MiSeq platform (Genomic Center, Azrieli Faculty of Medicine, BIU, Israel).

Following sequencing, microbial communities were analyzed using QIIME2⁴⁶. We used single-end sequences; sequence reads were demultiplexed by per-sample barcodes and Illumina-sequencing amplicon read errors were corrected by Divisive Amplicon Denoising Algorithm (DADA2)⁴⁷. A phylogenetic tree was generated. All analyses for mouse fecal samples were calculated based on a bacterial feature table containing features observed on samples containing at least 8,000 sequences. Alpha diversity (within sample diversity) parameters were calculated using the Faith's phylogenetic diversity⁴³. Beta diversity was analyzed using Jaccard similarity and Bray-Curtis dissimilarity. We also performed differential abundance testing (ANCOM)⁴⁴ which determines the features that are significantly differentially expressed between groups. The human microbiome samples in the learning and statistical analyses passed through the MIPMLP preprocessing pipeline²¹. In the human analyses, we merged taxa data at the genus level by using the mean procedure. We further applied log normalization on the samples.

Statistical analyses

We used IBM SPSS Statistics for Macintosh, version 25 (IBM Corp., Armonk, N.Y., USA). Chi-square and t-tests were used to compare behavioral and psychiatric variables between exposed individuals and controls, and Pearson and Spearman correlations tested associations among variables. To predict exposure and PTSD from the gut microbiota among adolescents, mothers and all subjects together, we used XGBOOST gb linear classification with the defaulting parameters¹⁴ (16S rRNA gene sequences) of mothers and adolescents together and separately. Data were split 50 times into training (80%) and test (20%) sets. Our metric was area under the curve (AUC). In addition, we also predicted the continuous CBCL from the microbiome by XGBOOST gb linear regression. The results were reported as an average of

50 runs similar to the classification. Our metrics were R^2 as well as Spearman's correlation coefficient (SCC). We have further calculated mother-adolescent microbial synchrony by the Euclidian distance of the log normalized amplicon sequence variants (ASVs) of the mother and her adolescent. High synchrony was defined as lower Euclidean distance, while low synchrony was defined as high mother-adolescent distance. A Kruskal-Wallis H test examined differences in Shannon's alpha diversity scores between groups in early childhood, and specifically between exposed with PTSD vs. exposed without symptoms vs. controls, and Mann-Whitney post-hoc tests were utilized. Finally, to test the direct and indirect paths from children's PTSD at early childhood to emotional problems in late adolescence via the late adolescent microbiome, two models were formulated. In the first, we conducted a path analysis using IBM SPSS Amos 25.0 (IBM Corporation, Armonk, NY). Child age and gender were controlled and missing data were handled using the maximum likelihood method, a state-of-the-art method for obtaining estimates of the parameters⁴¹. Indicators of model fit were: nonsignificant chi-square values; root mean square error of approximation (RMSEA) equal to or less than .06; and comparative fit index (CFI), with Tucker-Lewis index (TLI) values >0.95 considered good fit⁴⁵. To assess the significance of the mediation effects, we used a procedure recommended by Hayes⁴⁶ and calculated the 95% confidence intervals (CI) of the indirect effects based on 5,000 bias-corrected and accelerated bootstrapped samples. Indirect effects in which zero is not included in the 95% CI indicate a significant effect at $\alpha < .05$. To confirm that our findings were not affected by the missing data imputation, we ran the same linear mediation model using PROCESS macro for SPSS (v. 3.4.1)⁴⁶. PROCESS employs bootstrapping calculations, a nonparametric resampling procedure, which provides a powerful method of obtaining confidence limits for specific indirect effects⁴⁷. For this analysis, bias-corrected standard errors and confidence intervals were generated using 5,000 bootstrapped samples. Mediation is considered present when the CI for the estimation of the indirect effect does not contain zero. A second model predicted CBCL in late adolescence from PTSD diagnosis in early childhood and the microbiome composition utilizing three alternative Ridge regression models. The full model received input from both the microbiome and the one hot encoded psychopathology variable from early childhood (the 3 different groups: Exp-PTSD, Exp-ND and No-TR). Two alternative models were used: the first alternative model is a sub-model of the full one, shared the same weight for the microbial variables and contained no psychiatric variables and the second alternative model shared the same weights of the psychiatric variables and did not contain any microbial features. We compared the full model and the two sub-models separately by applying the F-test. In addition, in order to find the significant taxa of the model, we compared the models' coefficients to the coefficients of a shuffled taxa model without shuffling the psychiatric variables and the outcomes. Finally, we defined the taxa as significant with the highest percentile in absolute values.

Mouse phenotypic transfer experiment

Germ-free Swiss Webster mice (n=16) were housed in flexible plastic gnotobiotic isolators in the animal facility at the Azrieli Faculty of Medicine, Bar-Ilan University, with controlled temperature (22°C) and light cycle (12 h light and 12 h dark). The mice had free access to autoclaved food and water. Colonization was performed at six weeks old using samples from 8 Exp-ND and 8 Exp-PTSD children by resuspending human stool samples in sterile PBS. Each mouse was orally gavaged with 300 µL of a sample once a week for two weeks. After the first transplant, mice were moved to cages in the conventional room of the animal facility. Three weeks after the first transplant, fecal samples were collected, and anxiety-related behavior was examined using the elevated plus maze (EMP)²⁶. The mice were placed at the junction of an elevated four-arm maze in which two arms are open and two are enclosed. The number of times the mice entered each of the open/closed arms was recorded for 5 minutes and later coded by two coders who exceeded over 90% reliability on all videos. All animal procedures were approved by the Medicine IACUC Committee (permit 47-07-2020).

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Competing interests: The authors declare no competing interests

Author contribution: KY, ST, OS, YL, OK, and RF designed, performed, and interpreted the study and wrote the manuscript. ST, LM, OZS and OK performed and analyzed all the microbiome sequencing and analyses. OS and YL performed all the computational analysis and KY performed the behavioral data collection and analyses and RF supervised the data collection and analyses. ST and LM performed animal experiments and OK supervised them. RF conducts the longitudinal study.

Data availability: Sequencing data is currently being uploaded to QIITA/EBI and will be available upon acceptance. Behavioral data that support the findings of this study is available on request from the corresponding author RF. The data are not publicly available due to them containing information that could compromise research participant privacy.

Correspondence and requests for materials should be addressed to RF.

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Figures

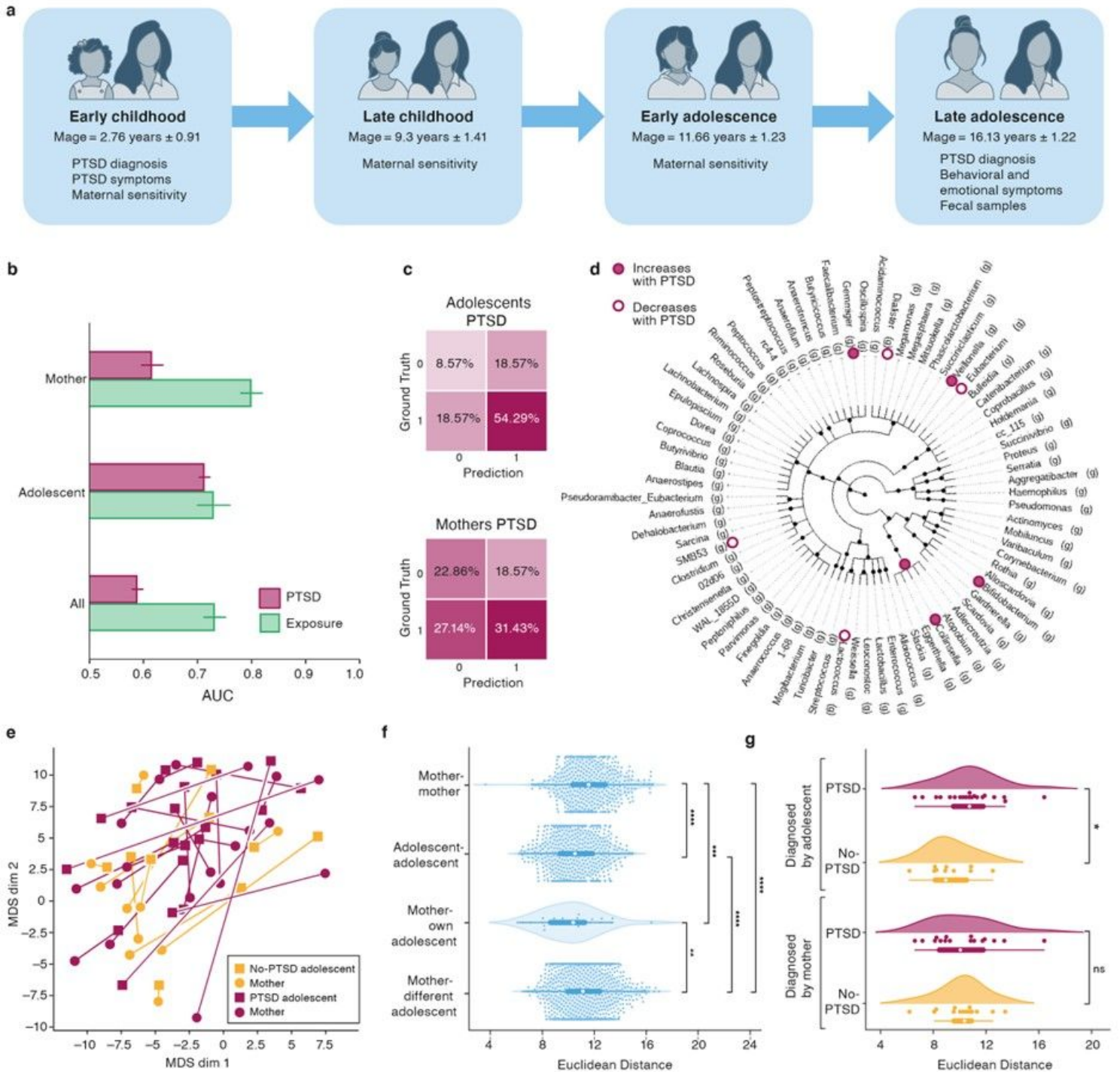


Figure 1

Trauma exposure and PTSD link with microbial composition, diversity and synchrony. a, Timeline and study parameters collected from early childhood to late adolescence. b, Trauma-exposure and PTSD predicted from gut microbial composition for all participants, only adolescents, and only mothers using XGBOOST gb linear classification with the default parameters. The results of learning are the area under the curve (AUC) of an average of 50 different runs on the external tests (20% of the data) (n=65; 31 mothers, 34 adolescents). c, Confusion matrix of predictions of the adolescents' PTSD model (upper) and the mothers' PTSD model. The x-axis represents the predictions (0-no PTSD and 1-PTSD). d, Taxa tree

represents the input microbiome of the adolescents' PTSD XGBOOST model. Big circles next to taxa represent taxa with significant feature importance according to the XGBOOST model. Empty circles indicate a decrease and filled circles indicate an increase in the bacterial abundance among PTSD youth. e, Illustrative representation of the Euclidian distance of the log normalized amplicon sequence variants (ASVs) of the mother and her own adolescent. High microbial synchrony is defined by smaller distances between dyads, while low microbial synchrony is defined as greater mother-adolescent distance. f, Comparing Euclidian distances between all mothers, all adolescents, mothers and their adolescents, and mothers and other adolescents. Squares denote children and circles denote mothers (n of mother-adolescent dyads =30). g, Comparing microbial synchrony between dyads with and without adolescent PTSD diagnosis and between dyads with and without maternal PTSD diagnosis. Pink denotes dyads in which the child suffers from PTSD, and yellow denotes dyads in which the child does not suffer from PTSD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

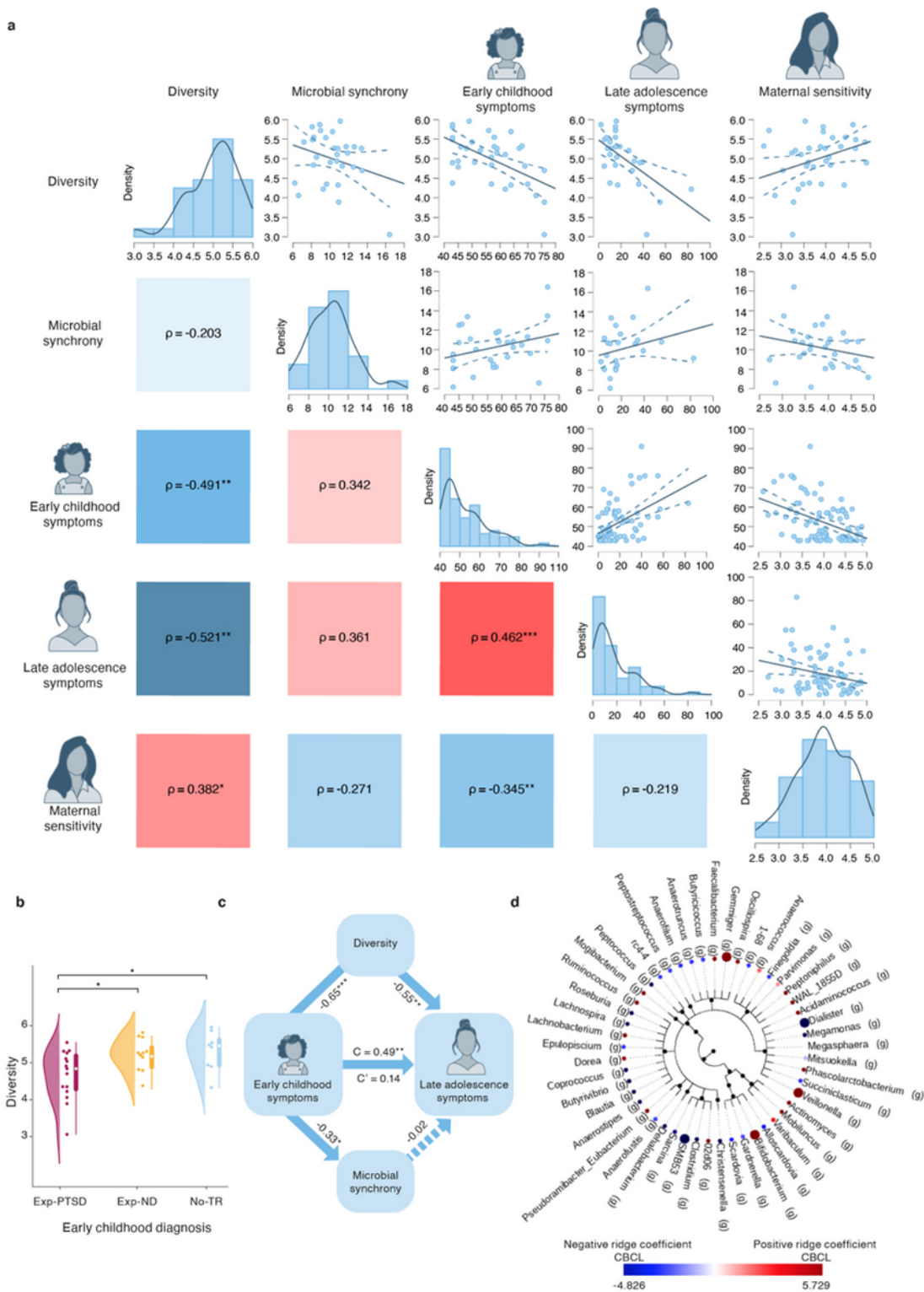


Figure 2

Gut microbiome is associated with post-traumatic symptomatology and sensitive caregiving. a, Bottom left corner: Spearman's correlation coefficient (ρ) between the study's main variables, colors represent negative (blue) and positive (red) correlations; upper right corner: scatterplots of main variables, the diagonal line present the linear trend and the dashed lines represent the 95% confidence interval around it; variable distributions are presented on the diagonal. b, Differences in adolescent alpha diversity across

groups based on early childhood PTSD (n=15 exposed with PTSD [Exp-PTSD], n=11 exposed with no PTSD or other diagnosis; [Exp-ND], n=8 not exposed with no PTSD or other diagnosis; [No-TR]). Kruskal-Wallis H test and Mann-Whitney post-hoc tests. c, Direct-Indirect paths from children's PTSD in early childhood to emotional problems in late adolescence via adolescents' microbiome with child age and gender controlled (n=84). Model parameters were examined with maximum likelihood estimation using the AMOS 21.0 program. Model fit: $\chi^2(1) = 0.08$, $p = 0.78$, CFI = 1.00, TLI = 1.60, NFI = 0.99, RMSEA < 0.001. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. d, Taxa tree represents the input microbiome of both the XGBOOST of the adolescents' prediction of PTSD and the input of the combined Ridge model of the PTSD diagnosis from early childhood and the microbiome from adolescence predicting psychological problems at adolescence. The big circles represent the bacteria that significantly contribute to the XGBOOST. The color represents the coefficient of the taxa in the Ridge model, such that high positive coefficients are marked in blue and low negative coefficients are marked in red. The big and dark taxa are consistent between the two models.

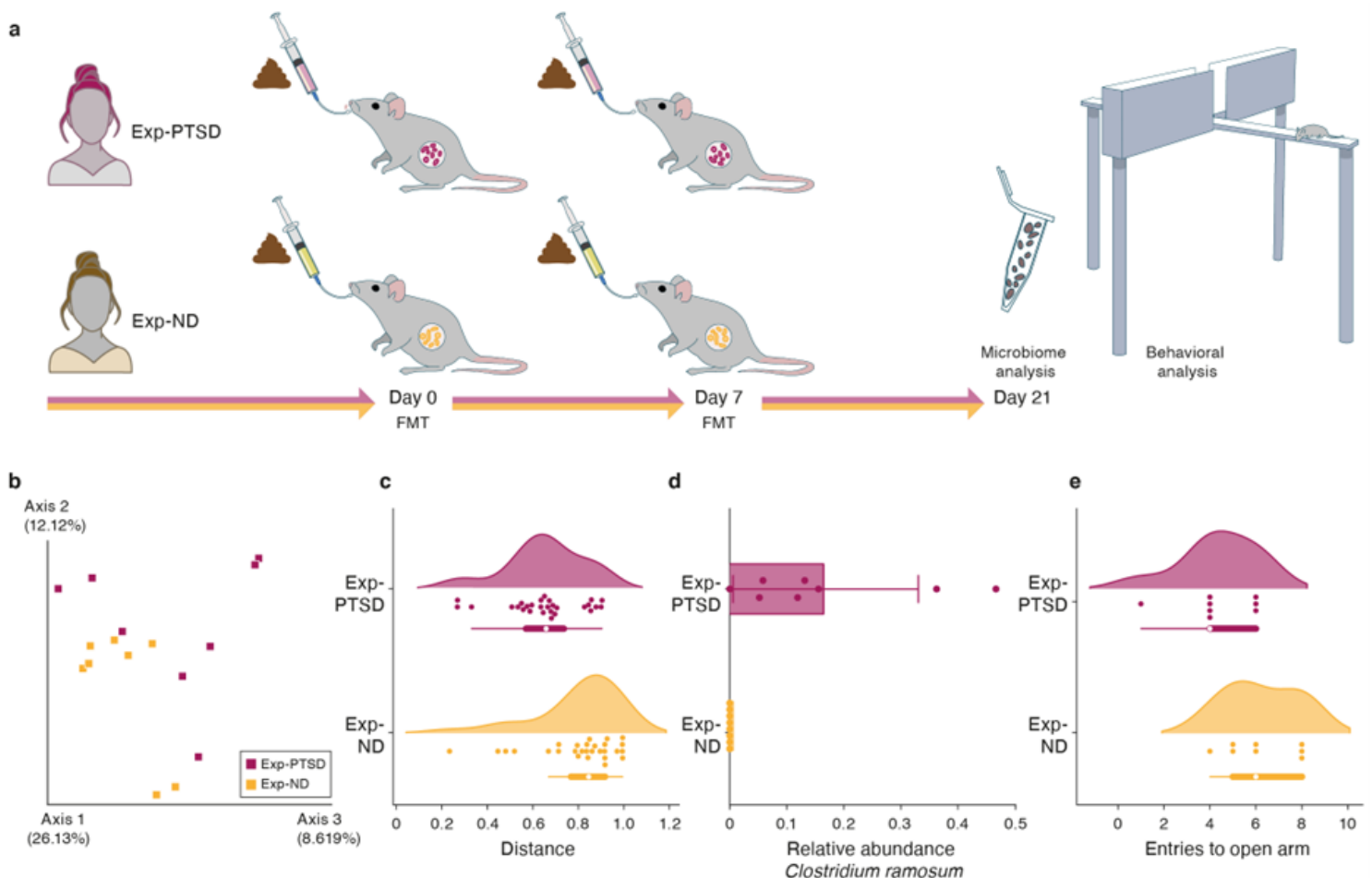


Figure 3

Causative role for the microbiome in the PTSD phenotype. a, The germ-free mouse model used to determine the role of the microbiome in PTSD. Germ-free mice received 2 fecal microbiota transplants (FMT) from war-exposed adolescents with (pink) and without (yellow) PTSD and three weeks later

anxious behavior was tested with an elevated plus maze behavioral assay. b, Principal coordinates analysis based on Jaccard distance showing a significant difference in beta diversity of the post-FMT mouse microbiota. c, Microbiome distance comparisons calculated using Bray–Curtis dissimilarities also showed a significant difference in post-FMT mouse microbiota. d, Relative abundance (%) at species-level of *Clostridium ramosum* in post-FMT mice (W=82). e, T-test between post-FMT mice with (n=8) and without (n=8) PTSD in the number of entries to the open arms at the EPM. *p<0.05, **p<0.01, ***p<0.001.

Supplementary Files

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- [Extendeddatafigure1.png](#)